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Letter to the Editor: Backbone resonance assignments of the 45.3 kDa catalytic domain of human BACE1

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Biological context

Alzheimer's disease (AD) is characterized by severe memory loss and neuronal cell death. It is estimated that over 4 million Americans have developed AD for which currently no cure is available. There are urgent needs for developing new therapeutics to improve patients' quality of life (Mintzer, 2003). AD is thought to be caused by the progressive brain accumulation of amyloidogenic $A\beta_{40,42}$ peptides into fibrillar aggregates and insoluble plaques (Vassar, 2002). Elucidating the molecular pathway involved in the generation of Aβ is the key for rational therapeutic approaches to lower Aβ concentration in AD. The Aβ peptide is generated by endoproteolysis of the large type I membrane protein called the β-amyloid precursor protein (APP). APP is processed by three types of protease activities: α-secretase (Buxbaum et al., 1998), β-secretase (Vassar, 2002) and γ-secretase (Wolfe et al., 1999); the molecular identities of which were only identified in recent years. The β-secretase was identified as a novel membrane-associated aspartic protease, β-site APP Cleaving Enzyme 1 (BACE1) (Vassar, 2002). Results from BACE1 knockout experiments indicated that BACE1 is responsible for the majority of the β-secretase activity (Luo et al., 2001). Importantly, the normal phenotype of BACE1 knockout mice suggested that BACE1 is dispensable for normal development and physiological functions *in vivo*, establishing BACE1 as an attractive drug target for the therapeutic intervention of AD.

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The human BACE1 protein contains 501 amino acids (Vassar et al., 1999), with a signal pre-peptide (residues 1–21) and a pro-peptide (residues 22–45) located N-terminal to the catalytic domain which resides between residues 46 and 460. An additional transmembrane domain and a cytosolic domain (residues 461–501) are located at the C-terminus of BACE1. The crystal structure of the BACE1 protease domain in complex with a transition-state inhibitor was determined at 1.9 Å resolution (Hong et al., 2000). The BACE1 catalytic domain forms a typical bilobal structure which is similar to pepsin and other aspartic proteases despite their low sequence conservations. The two conserved aspartic acids in the active site are located between the N- and C-terminal lobes. The active site is partially covered by a hairpin loop, also known as the 'flap'. The 'flap' is considered dynamic and important for protein-inhibitor interactions (Hong et al., 2000).

To further characterize this pharmaceutically important enzyme and for NMR-assisted drug discovery, we have initiated studies of the structure and dynamics of BACE1 using multidimensional NMR techniques. Here, we report backbone resonance assignments for the catalytic domain of human BACE1.

Methods and results

Details on the expression and purification of the human BACE1 protein will be presented elsewhere. Briefly, the human BACE1 protein (residues 14–454, including pre, pro and catalytic domains) was overexpressed in *E. coli* BL21(DE3) strain. BACE1 was insoluble and refolded from inclusion bodies

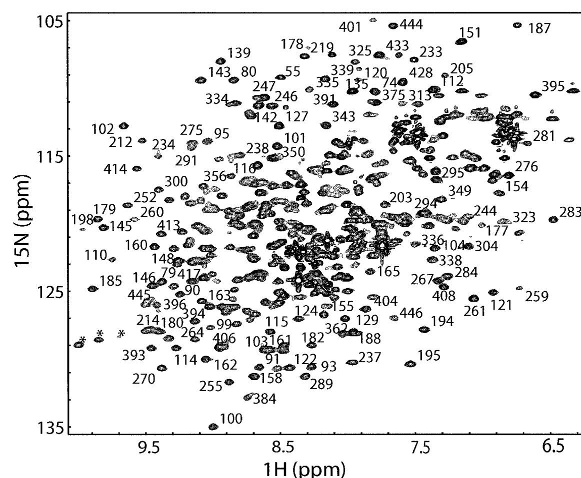

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of 0.9 mM [U-^{2}H,^{15}N,^{13}C]-labeled human BACE1 acquired at 600 MHz. Peaks labeled with * are from tryptophan side chain HNs. Selected peaks are labeled with their corresponding residue numbers.

that were treated with denaturant and detergent, and subsequently subjected to a refolding scheme. Refolded material was concentrated and applied to a Superdex-200 gel filtration column. The fractions with BACE catalytic activity were collected and combined. The protein solution was first applied to a Resource-Q column and subjected to a Superdex-200 column for further purification. The pre- and propeptides of BACE1 are auto catalytically removed to yield residues 48–454 of the catalytic domain with a molecular weight of 45,341 Daltons. Uniformly ^{2}H, ^{15}N and ^{13}C labeled BACE1 protein was exchanged into a phosphate buffer containing 75 mM KiPO4, pH 7.5, 150 mM NaCl and 10% D_2O for NMR studies. NMR experiments were performed at 25 °C on a Varian *INOVA* 600 MHz spectrometer. The following TROSY-based 3D NMR experiments were performed: HNCO, HNCA, HN(CO)CA, HN(CA)CO, CT-CACB(CO)NH, HNCACB (ProteinPack, Varian Inc.). Three 4D datasets, 4D TROSY-HNCACO, 4D TROSY-HNCOCA and 4D TROSY-HNCO$_{i-1}$CA (Tugarinov et al., 2002) were acquired to facilitate the assignment process. A 3D ^{15}N-edited NOESY-HSQC was also recorded. NMR data were processed with NMRPipe (Delaglio et al., 1995) and analyzed with NMRView (Johnson and Blevins, 1994) on a Silicon Graphics workstation.

Extent of assignments and data deposition

The 2D ^{1}H-^{15}N HSQC spectrum of human BACE1 is shown in Figure 1. Backbone resonances for 321 out of 386 assignable non-proline residues were assigned (83%). Nearly all the resolved peaks in the ^{1}H-^{15}N HSQC spectrum could be assigned. The unassigned residues are largely due to either missing cross peaks in the triple resonance data sets and/or degenerated chemical shifts, which prevented unambiguous assignment solutions. In addition, many unassigned residues located in turn and loop regions are likely experiencing either chemical exchange and/or conformational exchange. Given the relative high molecular weight of BACE1, the TROSY based 3D and 4D triple resonance experiments were essential to the assignment process. The ^{1}H, ^{15}N, and ^{13}C chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 8997.

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